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Behavioral changes in the brown planthopper, *Nilaparvata lugens*, mediated by melatonin

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Abstract

Background: Melatonin has been proved to exist and play importance roles in rice plant, such as biosynthesis and resistance. However, little is known about the function of melatonin in its monophagous pest, the brown planthopper.

Methods: In this study, we examined the effects of melatonin on the copulatory and locomotor behaviors of brachypterous and macropterous adult planthoppers by exposing them to melatonin, luzindole (a melatonin receptor antagonist), or a combination of melatonin and luzindole.

Results: A total of 68.7% of copulation events occurred at night in the control, while 31.2% occurred at night in the melatonin treatment, which led to a decrease in offspring. Brachypterous males were involved in mating events in the melatonin treatment but not in the other two treatments or the control. The daily locomotor pattern in the melatonin treatment was markedly different from that in the luzindole and melatonin and luzindole treatments. The total locomotor activities of the macropterous and brachypterous males exposed with melatonin were suppressed compared to those in the control. Melatonin significantly decreased the daytime and nighttime locomotor activities of macropterous females. In comparison, the activity of brachypterous females decreased slightly in the daytime but was more than double that of the control females at night.

Conclusions: Our results reveal that melatonin plays a role in the behaviors of brown planthoppers.

Keywords: Copulation, Locomotion, Luzindole, Rice, Pest control

Introduction

The detection of melatonin in the compound eyes of locusts (*Locusta migratoria*) represented the first confirmation of the existence of melatonin in insects (Vivien-Roels et al. 1984). Within insects, most of the melatonin is contained in the head, particularly the compound eyes (Bloch et al. 2013; Itoh et al. 1995a). In spite of the extremely low content, melatonin is indispensable for many processes in insects, such as embryonic

development, molting and reproduction (Vivien-Roels and Pévet 1993; Itoh et al. 1995b; Itoh and Sumi 1998; Klaus et al. 2000). Melatonin is widely known as a chronological pacemaker or “Zeitgeber” in signaling and plays several roles in circadian rhythms. Similarly to mammals, the circadian/behavioral changes due to melatonin were observed in insects (Vivien-Roels and Pévet 1993; Pandi-Perumal et al. 2006). Adding melatonin to the drinking water of house cricket, *Acheta domesticus*, led to reduced locomotor activities, with variation between day and night (Yamano et al. 2001). Melatonin in male cabbage looper moth (*Trichoplusia ni*) influenced its endogenous circadian regulation, with a high night peak even under continuous darkness (Linn et al. 1995). It was

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proven that female fruit flies, *Drosophila melanogaster*, had decreased mating speeds and oviposition rates after being injected with melatonin (Finocchiaro et al. 1988). The reproduction of pea aphid was also shown to be affected by melatonin treatments (Gao and Hardie 1997).

In addition to endogenous production within their bodies, insects can acquire additional melatonin from external sources (Brodbeck et al. 1998; Barberà et al. 2013; Iriti and Varoni 2016). For phytophagous insects, host plants can be sources of supplemental melatonin. Plants are known to have higher concentrations of melatonin than animals (Dubbels et al. 1995; Manchester et al. 2000). Mitochondria and chloroplasts have the capacity to synthesize and metabolize melatonin. The concentration of melatonin in rice seedlings can be as high as 300 pg/100 mg (Byeon and Back 2014). Therefore, our objective was to determine whether the brown planthopper, *Nilaparvata lugens*, a monophagous herbivore of rice, is influenced by melatonin.

As one of the most serious pests of rice (one of the major grain crops for half of the world's population (Khush 1999)), the brown planthopper causes considerable economic damage by feeding on rice plants and transmitting viruses associated with grassy stunt and ragged stunt diseases (Sōgawa 1982). These insects have developed resistance to selected pesticides (Wu et al. 2017; Diptaningsari et al. 2019). The brown planthopper shows wing dimorphism in both sexes: adults with fully developed wings, i.e., the macropterous form, and adults with truncated wings, i.e., the brachypterous form. Wing dimorphism is considered an example of successful evolution for migratory insects: the wings of macropterous planthoppers are used for migration and colonizing new rice fields, whereas brachypterous individuals mainly produce offspring (Xu and Zhang 2017).

In this indoor study, we tested the roles of melatonin on the copulatory and locomotor behaviors of both macropterous and brachypterous *N. lugens* adults. The objective was to determine whether melatonin could influence their activities. This research may contribute to defining ways to control pests in the field using benign methods. To do so, we investigated the possibility of increasing the melatonin concentration of the test insects. We also investigated the effect of luzindole in an effort to weaken the effects of melatonin.

Materials and methods

Insect rearing

The tested brown planthoppers were offspring of individuals collected in a rice field in Fuzhou, China, on 20 October 2017. They were reared indoors for at least 10 generations under the following conditions: a 14:10 photoperiod (5:00–18:59: LED lights used to simulate

daytime; 19:00–4:59: darkness to simulate nighttime, China standard time) and temperature of 26 ± 1 °C.

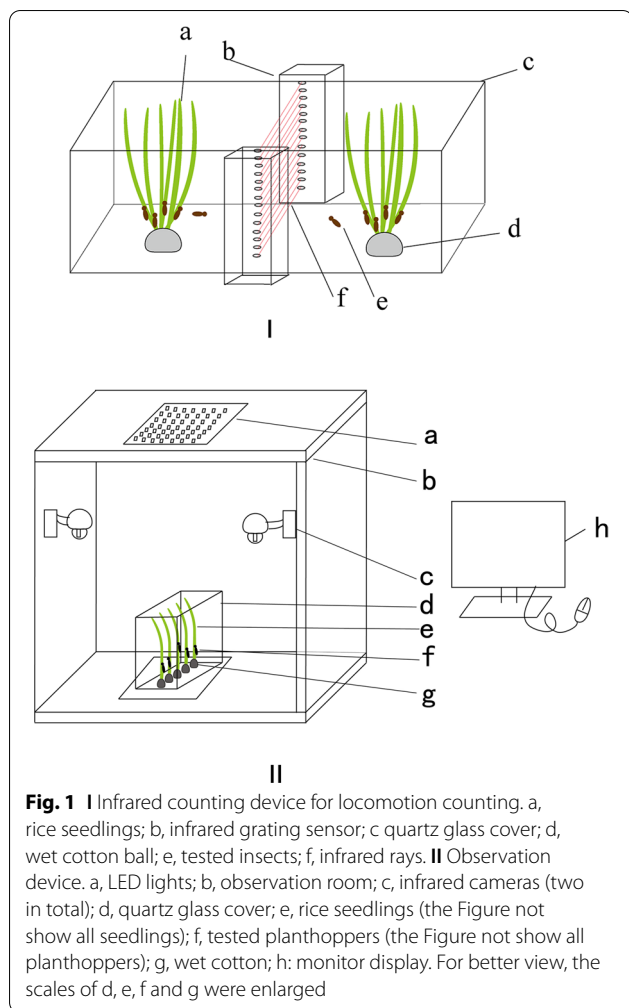
Artificially induced changes in the melatonin concentrations in the rice seedlings and *N. lugens*

To confirm that the rice roots could take up external melatonin and that the concentration of melatonin in the rice seedlings and planthoppers that fed on the seedlings could be influenced, more than 100 newly emerged *N. lugens* nymphs (i.e., those that had hatched in the preceding 12 h) were placed together and reared with 100 rice seedlings (10 days after sprouting) in each cage. The roots of the rice seedlings were soaked in one of the following four treatments (a total of 12 cages were used, with three replicates per treatment): (A) melatonin at 1×10^8 pg/ml, (B) luzindole at 1×10^6 pg/ml, (C) melatonin and luzindole at 1×10^8 pg/ml and 1×10^6 pg/ml (at a 1:1 ratio), and (D) distilled water as a control. The concentrations of melatonin and luzindole we used in the experiments were referred to the research of Gao and Hardie (1997) and Qiushi et al. (2015) confirmed by a series of preliminary analyses to be appropriate for the tests (data unpublished). The rearing conditions were the same as those described above.

The emerging adults as well as the rice seedlings (without roots) from each cage were collected from 23:00 to 24:00 h under dim light and immediately stored at -80 °C. For each cage, 100 mg of planthopper adults and 100 mg of rice seedlings were weighed and analyzed for melatonin concentration separately. Prior to the melatonin analysis, the planthoppers and rice seedlings were washed in 75% v:v ethyl alcohol to avoid contamination. The melatonin concentrations in the planthoppers and rice seedlings were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Insect MT ELISA KIT, Shanghai Enzyme-linked Biotechnology Co., Ltd., China) (Chegini et al. 1995; Li and Cassone 2015; Bagci et al. 2017).

Observations of locomotion by adults in various melatonin treatments

To accurately record locomotor activities, we designed a counting device (Fig. 1) comprised of two infrared grating sensors (one receiver and one transmitter), a rectangular quartz glass cover ($20 \times 8 \times 10$ cm, L \times H \times W; 1.0 mm in thickness) and a counter. The device created a grid of infrared rays between the two grating sensors. The interval between two adjacent infrared rays was 1.25 mm, which is shorter than the body width of adult planthoppers. Locomotor activity was recorded every time a planthopper moved/flew through the infrared grid, thereby cutting one or more infrared rays in the process. Dozens of final instar planthopper nymphs were collected from



the rearing room and separately kept alive on fresh rice seedlings until they molted into adults. The newly molted adults collected within a 12-h period were classified into four insect types: macropterous females (MF), macropterous males (MM), brachypterous females (BF) and brachypterous males (BM). Ten test individuals from the same group were put into the quartz cages (Fig. 1Ic) with 5- to 7-day-old rice seedlings with their roots wrapped in cotton (Fig. 1Ia, d). The cotton was kept wet with one of the following four liquids: (A) melatonin at 1×10^8 pg/ml, (B) luzindole at 1×10^6 pg/ml, (C) melatonin and luzindole at 1×10^8 pg/ml + 1×10^6 pg/ml (at a 1:1 ratio), and (D) distilled water as a control. The locomotor activities of the test individuals were recorded starting at 0:00 the day after eclosion and ending at 23:59 on the 6th day after eclosion. In each recording session, the number of locomotor activities and the time of each activity were recorded. The recordings in which one or more of the test individuals died were discarded. There were 48 recordings [4 insect types \times (3 treatments + 1 control) \times 3

replicates] in which there was no mortality of the tested individuals.

Observations of copulation among adults and comparison of offspring in various melatonin treatments

In this experiment, we made a device to observe the copulation activities of planthoppers. As shown in Fig. 1II, the observation device contained two high-speed infrared cameras (1/4 Sony CCD, 24 \times zoom, fast automatic focusing, with cradle control) positioned on the opposite sides of the observation room (1.2 m \times 1.2 m \times 1.2 m). To reduce outside noise and other interferences, the walls were soundproofed with cotton pressed between two wooden planks (each 5 cm thick), and the interior of the room was covered with a black deadener. LED lights were positioned at the top of the observation room. The 4th- or 5th-instar planthopper nymphs were collected from the original rearing cages and kept alive separately with fresh rice seedlings until they underwent metamorphosis into adults. The newly morphed adults (i.e., those that had emerged within 12 h) were collected. Five couples of virgin brachypterous adults and 5 couples of virgin macropterous adults were placed together in a quartz glass cage (see Fig. 1II d) with 10 rice seedlings (5–7 cm) placed equidistantly apart. The roots of the seedlings were wrapped in cotton, and as mentioned above, the cotton was kept wet by one of the four treatment solutions. The observations began at 0:00 on the day after eclosion, and the mating behaviors were recorded for 10 days by the cameras, with the recordings being stored in a computer. The data collected from the recordings included the specific time and the duration of each copulation event, which was defined as the time when the genitals of the two sexes were connected, ending with their separation. The copulation types included a couple of macropterous planthoppers (type I), one macropterous male and one brachypterous female (type II), a couple of brachypterous planthoppers (type III), and one brachypterous male and one macropterous female (type IV). In the observation room, the environmental conditions were maintained as described above. There was no mortality in any of the 12 observation groups during the course of the experiment.

Following the above experiment, the mated females remained in the cages to oviposit eggs on the rice seedlings. Thereafter, the planthoppers were removed from the observation cages, and the ten rice seedlings in each cage were kept alive until no more offspring emerged. Every day we checked and counted the new emerged offsprings.

Statistical analysis

The concentrations of melatonin in the planthoppers and rice seedlings and the hourly, daily and total numbers

of locomotor activities were analyzed using one-way ANOVA followed by an LSD multiple range test. The data satisfied the normality and homogeneity of variance requirements, no transformation was needed. Repeated measures analyses of variance was used to determine whether the variation over the 6-day assessment period was significant (within-subject variation) and if these results significantly differed among treatments, sexes and wing types (between-subject variation), the sphericity assumption was satisfied ($P > 0.05$).

Regarding the copulation and offspring data, except for the duration of mating, the other data satisfied assumptions of normality and homogeneity of variance; therefore, no transformation was needed. For the data on the copulation ratio in darkness and number of offspring, one-way ANOVA was performed, followed by an LSD multiple range test. The data were analyzed using SPSS 19.0 software, Microsoft Excel 2003 and OriginPro 8.

Results

Rice seedlings and brown planthoppers display different changes in melatonin contents under various treatments

As Table 1 shows, when compared to the control and luzindole treatments, the concentrations of melatonin in planthoppers and rice seedlings were significantly increased by the external melatonin ($P < 0.05$). In the melatonin & luzindole treatment, the concentration of melatonin was significantly higher than that in the luzindole treatment ($P < 0.05$). According to these results, we concluded that external melatonin application increased the melatonin concentration in the planthoppers and rice seedlings. This conclusion justified the following tests.

Locomotor activities of each type of adult planthoppers in the three treatments displayed varied changes

As the results of the repeated measures ANOVAs show (Table 2), within subjects, the number of locomotor activities differed significantly between the 6 observation days ($P = 0.043$); however, the corresponding number of daily

nighttime activities did not differ significantly ($P = 0.082$). Sex was the main factor that significantly influenced both the daily ($P = 0.003$) and nightly ($P = 0.014$) number of activities. Between subjects, the daily number of activities was significantly different among the three treatments ($P = 0.024$), whereas the number of nighttime activities per day was not ($P = 0.147$). With the exception of the sex \times wing type interaction ($P = 0.007$), none of the two- or three-way interactions was significant ($P > 0.05$).

In control, the mean number of total locomotion events of macropterous male adults over 6 days (1290.0 ± 11.94) was significantly higher than female adults (macropterous, 426.7 ± 23.03 ; brachypterous, 480.7 ± 19.36) and brachypterous male adults (786.7 ± 11.22). For all the four types of brown planthoppers, the total (6-day) number of activities in the luzindole treatment and melatonin & luzindole treatment (483.9 ± 7.89) were significantly higher than that in the control. When exposed with melatonin, the total events of macropterous female and male were decreased, while increased in brachypterous female and male (Table 3).

Like Fig. 2 shows, for both macropterous and brachypterous females, the daily locomotor activities in the control increased, peaking on the 3rd day, and then decreased progressively (Fig. 2a, c). In the control, the number of locomotor activities of macropterous males peaked on day 2, and then decreased over time (Fig. 2b). As to the brachypterous males, the daily locomotor activities decreased from days 1 to 6 (Fig. 2d). When exposed with melatonin, the activity values of the macropterous females on days 1, 2, 3, 5 and 6 were significantly lower than the values in the control, and the peak was delayed until day 4, at which time the value was significantly higher than that in the other treatments (Fig. 2a). For the brachypterous females, the number of daily activities in the melatonin treatment was lowest on day 2 and generally higher in the second half of the recording period (Fig. 2c). The macropterous males in the melatonin treatment displayed lower number of activities than that in the control on days 1 and 2 and equal to that in the control on days 3–6, the highest value was found on day 3 (Fig. 2b). The daily number of brachypterous male activities exposed with melatonin increased significantly on days 3 and 6 increased significantly compared to that in the control (Fig. 2d). The number of activities of macropterous female in the luzindole and melatonin & luzindole treatments increased with each passing day, with the highest value being found on the 6th day. There was no significant difference in the activity values recorded on the same day between the two treatments. The number of activities in the luzindole and melatonin & luzindole treatments differed significantly from that in the control only on days 5 and 6 (Fig. 2a). In the first 3 days of

Table 1 Concentrations of melatonin in planthopper and rice seedlings under the four treatments

Treatment	Melatonin in planthoppers (mean \pm SE, pg/100 mg)	Melatonin in rice seedlings (mean \pm SE, pg/100 mg)
Control	19.01 \pm 0.042b	230.06 \pm 16.510b
Melatonin	21.46 \pm 0.525a	265.91 \pm 1.231a
Luzindole	18.29 \pm 0.574b	227.50 \pm 4.955b
Melatonin & luzindole	22.02 \pm 0.154a	270.12 \pm 6.345a

Different letters within the same column of same object represent significant differences, LSD, $P < 0.05$

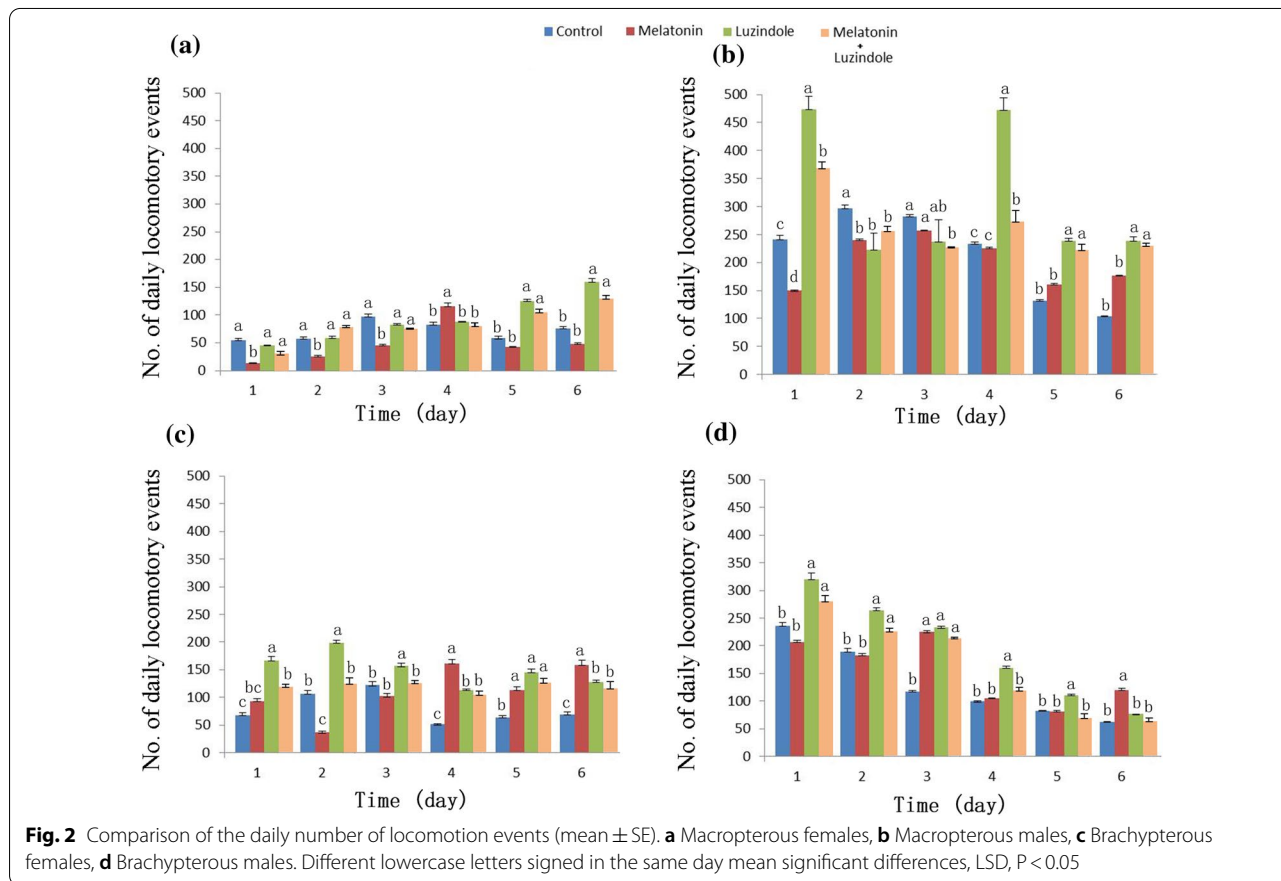
Table 2 Results of repeated-measures ANOVAs comparing the daily number of locomotion event recorded during the course of the enclosure experiment

Source of variation	Daily locomotion events			Daily night locomotion events		
	df	F-value	P-value	df	F-value	P-value
Within subject variation						
Day	5, 20	2.682	0.043	5, 20	2.043	0.082
Day * treatment	10, 42	0.992	0.443	10, 42	1.590	0.123
Day * sex	5, 20	4.675	0.003	5, 20	3.050	0.014
Day * wing type	5, 20	1.612	0.185	5, 20	2.756	0.024
Day * treatment * sex	10, 42	1.218	0.302	10, 42	1.061	0.398
Day * treatment * wing type	10, 42	0.958	0.468	10, 42	1.250	0.270
Day * wing type * sex	5, 20	0.372	0.805	5, 20	2.433	0.042
Day * treatment * sex * wing type	10, 42	0.674	0.694	10, 42	0.635	0.772
Between subject variation						
Treatment	2, 24	4.397	0.024	2, 24	2.081	0.147
Sex	1, 24	30.703	<0.001	1, 24	23.623	<0.001
Wing type	1, 24	1.083	0.309	1, 24	0.671	0.421
Treatment * sex	2, 24	0.332	0.721	2, 24	0.289	0.751
Treatment * wing	2, 24	0.496	0.615	2, 24	1.041	0.368
Sex * wing	1, 24	10.466	0.004	1, 24	8.771	0.007
Treatment * sex * wing type	2, 24	0.438	0.650	2, 24	0.248	0.783

Table 3 Comparison of total locomotion events over 6 days (mean ± SE)

Treatment	Macropterous females	Macropterous males	Brachypterous females	Brachypterous males
Melatonin	291.3 ± 10.62cD	1211.0 ± 41.81bA	665.3 ± 23.34bC	921.0 ± 8.45bB
Luzindole	560.0 ± 7.69aC	1885.3 ± 29.14aA	909.3 ± 14.62aB	1164.3 ± 15.66aB
Melatonin & luzindole	483.9 ± 7.89abD	1572.1 ± 33.02aA	713.7 ± 16.14abC	978.3 ± 16.32abB
Control	426.7 ± 23.03bC	1290.0 ± 11.94bA	480.7 ± 19.36bC	786.7 ± 11.22cB

Different lowercase letters signed in the same column mean significant differences, LSD, $P < 0.05$; different capital letters signed in the same row mean significant differences, LSD, $P < 0.05$



brachypterous female observations, the number of daily locomotor activities in the luzindole treatment was significantly higher than that in the other treatments, with the peak occurring on day 2. When exposed both with melatonin and luzindole, the daily number of activities dropped and remained stable over time (Fig. 2c). For the macropterous males, in the luzindole treatment, the extreme values were observed on days 1 and 4, which were significantly higher than the values in the other treatments on the same day. Similarly, in the melatonin and luzindole treatment, the two highest values occurred on days 1 and 4 (Fig. 2b). For the brachypterous males, the downward trend was also evident in the luzindole

and melatonin & luzindole treatments, wherein the daily numbers were significantly higher than in the control for the first 3 days, beyond which the differences were reduced (Fig. 2d).

The daily night proportions of locomotion events of the macropterous females, macropterous males, brachypterous females and brachypterous males were showed in Fig. 3. For the macropterous females, the proportions of activities on the first artificial night were similar, with values of approximately 0.7. Subsequently, the proportion in the control decreased over time and reached its lowest value on day 5 ($P < 0.3$) before rising again on day 6. After the first day, the proportion of nighttime activities

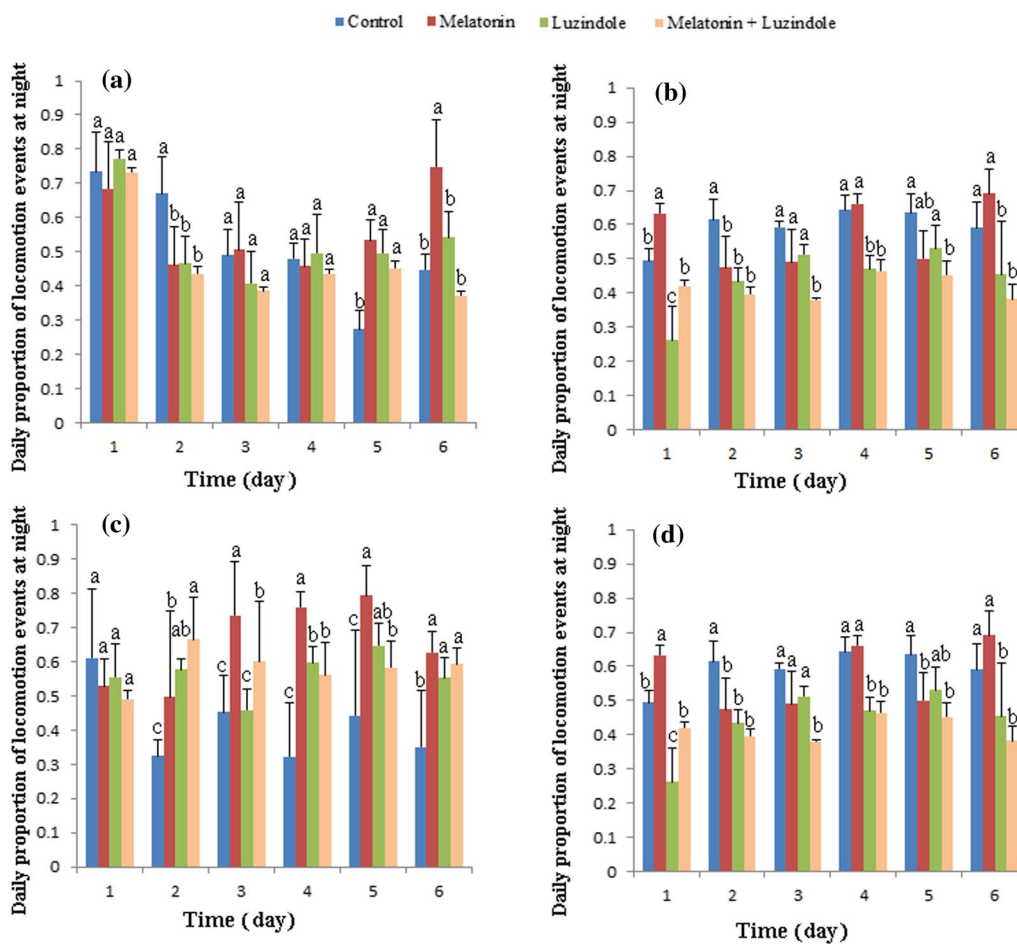


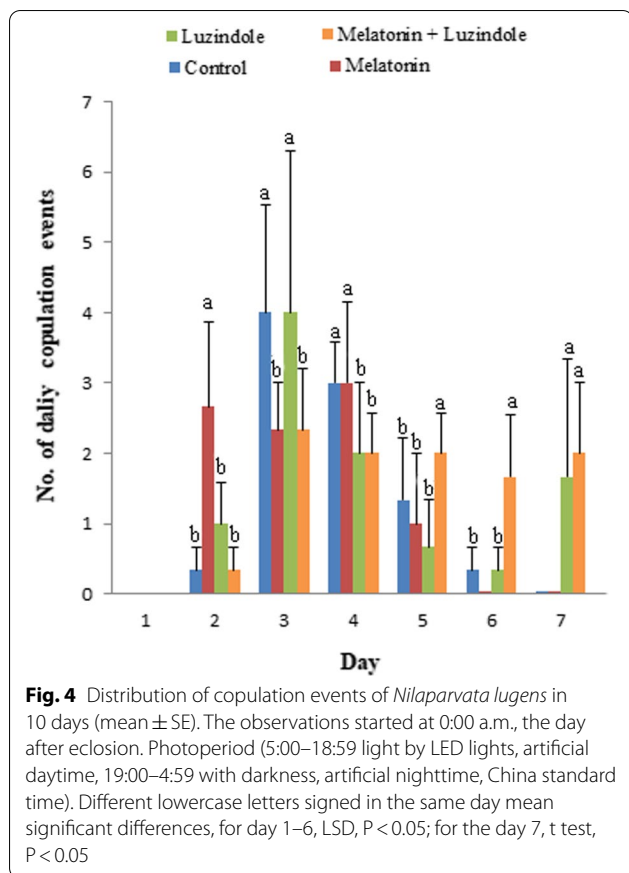
Fig. 3 Daily proportion of locomotion events at night (mean ± SE). (a) Macropterous females, (b) Macropterous males, (c) Brachypterous females, (d) Brachypterous males. Different lowercase letters signed in the same day mean significant differences, LSD, P < 0.05

in the luzindole and melatonin & luzindole treatments decreased and then fluctuated, with values of approximately 0.5 for the next 5 days. In the melatonin treatment, the proportion of nighttime activity from days 2–4 fluctuated, with values of approximately 0.5, before rising sharply in day 6 (Fig. 4a). In the control, the proportion of activities of the brachypterous females occurring at night was above 0.5 on day 1; this value then dropped and fluctuated below the 0.5 level for the remaining 5 days. On each observation day, the brachypterous females in the three treatments tended to move more at night than during the day (except for day 3 in the luzindole treatment, when the value fell to just below 0.5). Moreover, the brachypterous females exposed with melatonin displayed much higher activity at night than during the day, particularly on days 3, 4 and 5, when the proportions were higher than 0.7 (Fig. 3c). In the two luzindole treatments of macropterous males, the proportion of daily activities at night was ≤ 0.5 during the 6-day observation

period, whereas in the control, the corresponding value was ≥ 0.5 . By comparison, in the melatonin treatment, the daily proportion of activities fluctuated in a range from 0.45 to 0.7 (Fig. 3b). As to the brachypterous males, in the control, the luzindole treatment and melatonin & luzindole treatment, the mean proportion of daily nighttime activities fluctuated slightly, with values of approximately 0.5, whereas for the brachypterous males exposed with melatonin, the proportions were less than 0.5 in the first 5 days but increased to more than 0.6 on the last day (Fig. 3d).

Copulatory activities and offspring

A total of 114 copulation events were recorded in the twelve observation cages, with 27 events occurring in both the control and melatonin treatments, 29 events in the luzindole treatment, and 31 events in the melatonin and luzindole treatment (Table 4).



According to our results, copulation occurred during both artificial daytime (5:00–18:59) and artificial nighttime (19:00–4:59) in all four treatments. However, the temporal distribution of the copulation events varied. In the control treatment, planthoppers tended to mate during artificial nighttime, with a mean ratio of 68.7% (Table 4). In contrast, when melatonin was exposed in the cage, the adults preferred to mate during the day, only 31.2% of the copulation events occurring during the artificial nighttime (Table 4). In the luzindole treatment, the distribution of copulation events was relatively uniform between daytime and nighttime, with an average of 45.8%, which was higher than that in the melatonin treatment ($P < 0.05$) but still significantly lower than that in the control ($P < 0.05$) (Table 4). In the melatonin & luzindole treatment, 41.5% of the copulation events occurred at night, which was quite close to the percentage in the luzindole treatment but significantly higher than that in the melatonin treatment (Table 4).

In all four treatments, the first copulation events were observed on the second of ten observation days. For the control and the luzindole treatment, the peak of the copulation events occurred on the 3rd

Table 4 The copulation and reproduction variables of *N. lugens* (mean ± SE)

Treatment	Copulation ratio at night (%) [*]	Number of offspring	Type I		Type II		Type III		Type IV	
			Copulation events	Duration (s)	Copulation events	Duration (s)	Copulation events	Duration (s)	Copulation events	Duration (s)
Control	68.7 ± 16.13a	437.3 ± 41.22a	6.3 ± 1.33	125.86 ± 5.692	2.0 ± 0.58	106.33 ± 11.271	0	0	0	–
Melatonin	31.2 ± 7.93c	373.3 ± 46.45b	5.7 ± 1.45	132.24 ± 6.609	2.0 ± 0.00	132.20 ± 8.997	1.0 ± 0.58	76.33 ± 7.311	0.7 ± 0.67	172.5 ± 37.500
Luzindole	45.8 ± 12.15b	340.3 ± 23.55c	8.3 ± 2.60	124.96 ± 5.026	1.3 ± 0.33	143.00 ± 17.412	0	–	0	–
Melatonin & luzindole	41.5 ± 8.93b	331.7 ± 37.62c	8.6 ± 0.88	117.04 ± 2.663	2 ± 0.58	133.83 ± 4.191	0	–	0	–

^{*}Copulation ratio in darkness: Number of copulation events in artificial nighttime (19:00–4:59)/total number of copulation events. Different letters within the same column represent significant differences, LSD, $P < 0.05$

observation day (Fig. 4). In the melatonin treatment, the peak spread over the 2nd, 3rd and 4th days. When the adults were exposed with both melatonin and luzindole, the relationship looked like a trapezoid: from the third to the 7th observation day, the average copulation events were high and fluctuated between 2 and 3 (Fig. 4).

Despite ensuring an equal number of brachypterous and macropterous planthoppers (5 couples of newly emerged brachypterous planthoppers and 5 couples of newly emerged macropterous planthoppers), Type I (macropterous planthopper copulation) matings dominated the copulation events in all four treatments (6.3 events in the control, 5.7 events in the melatonin treatment, 8.3 events in the luzindole treatment and 8.6 events in the melatonin and luzindole treatment) (Table 4). Interestingly, different types of copulation events were found in the melatonin treatment (on average, 1.0 Type III event and 0.7 Type IV event), while no mating events included brachypterous males in the other three treatments.

As Table 4 shows, compared to the control, the numbers of emerged offspring in the three treatments were all significantly reduced. Among them, the offspring number in the melatonin (373.3) treatment was much higher than that in the luzindole (340.3) and melatonin and luzindole (331.7) treatments. There was no significant difference between the two luzindole treatments.

Discussion

Our study proved that luzindole had no serious effect on the concentration of melatonin in planthoppers or rice seedlings. As reported previously, luzindole is a high-affinity melatonin receptor antagonist. It can inhibit the binding of melatonin and melatonin receptors, which weakens melatonin activity (Sugawara et al. 1998). Luzindole has been used in some insect studies as a melatonin receptor antagonist (Richter et al. 1999; Klaus et al. 2000; Qiushi et al. 2015).

As all the tested *N. lugens* adults were newly emerged virgins and were fed fresh rice seedlings (food was not limited), even the macropterous females could copulate (Chen and Chen 1980). The courtship behaviors of brown planthopper are consistent with a “call-fly” strategy, wherein a male moves frequently and creates a specific vibrational signal. When a female receives the signal, it stays still and responds with another specific vibrational signal. The male receiving the female’s signal moves closer and finds the female (Toshihide and Shoziro 1974; Butlin 1993; Fu et al. 1997). In the process of courtship, males engage in more locomotor activities than females, and courtship typically occurs during the night, as our study indicated. In our study, the

males moved much more than the females and displayed more activities at night than during the day, regardless of wing type (macropterous or brachypterous). In the three treatments, especially the luzindole and luzindole and melatonin treatments, the activities of the males in the daytime increased remarkably when compared to those in the control. We hypothesize that increased activity in the daytime increases the risk of hunting by enemies and results in increased energy expenditure, among other consequences, which may adversely impact male survival. Further studies will be required to test the validity of our hypothesis.

In our mating and oviposition observations, the luzindole and melatonin & luzindole treatments both minimized the difference between the copulation ratio in the artificial daytime and nighttime and significantly decreased the number of offspring, which were all significantly different from the melatonin treatment and the control. These results confirmed that melatonin played a role in the reproduction of *N. lugens* by binding to melatonin receptors (yet to be found) and that luzindole suppressed that binding. However, for the brown planthoppers, melatonin might not have to bind to receptors. The possibility that melatonin works independently of a receptor cannot be excluded. Quite a few nonreceptor-mediated effects of melatonin have been reported, with free radical scavenging being a typical example affecting female reproduction (Tan et al. 2000; Tamura et al. 2014; Zhao et al. 2017). In males, melatonin is a potent inhibitor of androgen production in gonadal cells and works in a membrane receptor-dependent manner (Wang et al. 2018).

When organized as single mating pairs, brachypterous males and macropterous males were equally capable of mating with any females (Oh 1979). However, at sexual maturity, macropterous males tend to be more successful than brachypterous males at attracting females in one-on-one competitions (Novotný 1995). In our study, when both brachypterous and macropterous adults were present in the mating experiment, the brachypterous males failed to mate, except in the melatonin treatment. We suggest that competition may be a factor in the success of males with one wing type versus the other and that melatonin may influence the behavior of the two types of males, thus altering the level of competition between them.

The mechanisms behind such an influence of melatonin remain unknown. However, it has been shown that melatonin can be detected in several tissues of invertebrates, such as the brain and retina (Vivien-Roels and Pévet 1993). It is possible that melatonin may act similarly to serotonin (5-hydroxytryptamine). Serotonin is a monoamine neurotransmitter in insects involved in

the modulation of various physiological and behavioral processes, such as olfaction, feeding, vision, aggression, sleep and circadian rhythms (Osborne and Neuheff 1974; Blenau and Thamm 2011; Ellen and Mercer 2012; Huser et al. 2012). For instance, serotonin is highly influenced by the circadian clock and can increase the sexual responses of males to females in *Bombyx mori* (Bloch et al. 2013; Hill et al. 2003).

This study proved that melatonin plays a role in the locomotor activities and reproduction of brown planthoppers. One of the original aims of this study was to estimate the potential value of melatonin for planthopper control in rice fields. Most insect species are light-sensitive, and their activities are rhythmic and influenced by day–night length variation (Jennersten and Morse 1991; Somanathan et al. 2007; Shi et al. 2017). As a “zeitgeber” and photoperiodic signal messenger (Vivien-Roels and Pévet 1993; Pandi-Perumal et al. 2006), melatonin could be used to control populations of insect pests in the field. Melatonin can be purchased at a reasonable cost and is readily available, making it potentially useful in pest control systems for planthoppers (Mura et al. 2017; Pool et al. 2018). To advance this potential, further research on the mechanisms underlying the effects of melatonin on *N. lugens* and rice plants as well as its influence on other organisms, such as insect predators, is required.

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Authors' contributions

Conceptualization: LS and ZZ; methodology: LS, JZ and LQ; validation: LS, JZ and LQ; formal analysis: LS and LQ; investigation: LS, LQ and ZX; resources: ZZ; data curation: ZX; writing—original draft preparation: LS; writing—review and editing: LS, JZ and ZZ; supervision: ZZ; project administration: LS; funding acquisition: ZZ. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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